

**KEY WORDS**

plasma; goat; pharmacokinetics

**REFERENCE**

van Baal, J.M.; van Leeuwen, M.B.; Schouten, T.J.; De Abreu, R.A. Sensitive high-performance liquid chromatographic determination of 6-mercaptopurine, 6-thioguanine, 6-mercaptopurine riboside and 6-thioguanosine in biological fluids, *J.Chromatogr.*, **1984**, 336, 422–428.

**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** Plasma. Filter (Amicon Model MM 302, type PM 10 Diaflo membrane) plasma while centrifuging at 8000 g for 3 min, inject a 20–100  $\mu$ L aliquot of the ultrafiltrate. Urine. Inject an aliquot directly.

**HPLC VARIABLES**

**Column:** 150  $\times$  4 Nucleosil 5C8

**Mobile phase:** 50 mM pH 7.0 citrate-phosphate buffer (Buffer was 9.61 g  $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$  and 1.33 g citric acid monohydrate in 1 L water, pH 7.0.)

**Flow rate:** 1.4

**Injection volume:** 20–100

**Detector:** UV 343

**CHROMATOGRAM**

**Retention time:** 5.7

**Limit of quantitation:** 200 ng/mL

**OTHER SUBSTANCES**

**Extracted:** 6-mercaptopurine

**Simultaneous:** allopurinol, azaguanine, guanine, oxipurinol, uric acid

**Noninterfering:** adenine, 2-amino-6-methylthiopurine, aspirin, benzbromarone, caffeine, diazepam, dihydralazine, dipyridamole, fluorouracil, hypoxanthine, methotrexate, procarbazine, propranolol, spironolactone, sulfamethoxazole, sulfapyrazone, theophylline, thiouric acid, thioxanthine, trimethoprim, xanthine

**KEY WORDS**

plasma; ultrafiltrate

**REFERENCE**

Breithaupt, H.; Goebel, G. Quantitative high pressure liquid chromatography of 6-thioguanine in biological fluids, *J.Chromatogr.Sci.*, **1981**, 19, 496–499.

# Thiopental

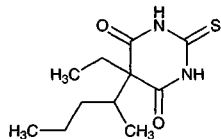
**Molecular formula:**  $\text{C}_{11}\text{H}_{18}\text{N}_2\text{O}_2\text{S}$

**Molecular weight:** 242.34

**CAS Registry No.:** 76-75-5, 71-73-8 (Na salt)

**Merck Index:** 9487

**Lednicer No.:** 1 274

**SAMPLE**

**Matrix:** blood

**Sample preparation:** Precipitate 100  $\mu$ L serum with 200  $\mu$ L 10  $\mu$ g/mL IS in MeCN, centrifuge at 12000 g for 5 min. Inject a 50  $\mu$ L aliquot of the supernatant.

**HPLC VARIABLES**

**Guard column:** 4  $\times$  4 5  $\mu$ m LiChroCART LiChrospher 60 RP Select B

**Column:** 125 × 4 5 µm LiChroCART LiChrospher 60 RP Select B

**Mobile phase:** MeCN:buffer 50:50 (Buffer was 25 mM pH 3.0 triethylammonium phosphate containing 2% MeCN.)

**Flow rate:** 2

**Injection volume:** 50

**Detector:** UV 283

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**CHROMATOGRAM**

**Retention time:** 3.46

**Internal standard:** thiobutabarbital (2.70)

**Limit of detection:** 230 ng/mL

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**KEY WORDS**

serum

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**REFERENCE**

Hannak,D.; Scharbert,F.; Kattermann,R. Stepwise binary gradient high-performance liquid chromatographic system for routine drug monitoring, *J.Chromatogr.A*, **1996**, 728, 307–310.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Mix 250 µL plasma with IS and 400 µL MeCN, vortex, centrifuge. Inject a 20 µL aliquot of the clear supernatant.

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**HPLC VARIABLES**

**Column:** C18 reverse phase

**Mobile phase:** MeOH:0.5% tetrabutylammonium phosphate in water 50:50

**Injection volume:** 20

**Detector:** UV 265

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**CHROMATOGRAM**

**Internal standard:** methohexital

**Limit of detection:** 100 ng/mL

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**KEY WORDS**

sheep; plasma; pharmacokinetics

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**REFERENCE**

Upton,R.N.; Huang,Y.F.; Grant,C.; Gray,E.C.; Ludbrook,G.L. Myocardial pharmacokinetics of thiopental in sheep after short-term administration: Relationship to thiopental-induced reductions in myocardial contractility, *J.Pharm.Sci.*, **1996**, 85, 863–867.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Buffer serum to pH 5.6 with 100 mM acetate buffer, extract with hexane. Remove the hexane and extract it with 250 mM NaOH. Neutralize the aqueous layer with phosphoric acid and inject an aliquot.

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**HPLC VARIABLES**

**Column:** µBondapak C18

**Mobile phase:** MeCN:0.1 mM pH 4.2 phosphate buffer 43:57

**Flow rate:** 1

**Detector:** UV 195

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**CHROMATOGRAM**

**Limit of detection:** 10 ng/mL

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**OTHER SUBSTANCES**

**Extracted:** methohexital

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**KEY WORDS**

serum; pharmacokinetics

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**REFERENCE**

Hudson,R.J.; Stanski,D.R.; Burch,P.G. Pharmacokinetics of methohexital and thiopental in surgical patients, *Anesthesiology*, **1983**, *59*, 215–219.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 2 mL Serum + 1 mL buffer, vortex, add 10 mL n-butyl chloride containing 10 µg/mL barbital and 4 µg/mL thiamylal, extract vigorously for 3 min, centrifuge at 3000 g for 5 min. Remove the upper organic layer and add it to 100 µL 450 mM NaOH, extract vigorously for 3 min, centrifuge for 10 min or until lower aqueous phase is clear, inject a 15 µL aliquot of the lower aqueous phase. (Soak glassware in 1 M HCl overnight, rinse with water, dry. Buffer was prepared from equal volumes of 22 g/L KH<sub>2</sub>PO<sub>4</sub> and 18 g/L Na<sub>2</sub>HPO<sub>4</sub>, pH 6.6 ± 0.2.)

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**HPLC VARIABLES**

**Column:** 125 × 4.6 5 µm C-18 (Perkin-Elmer)

**Mobile phase:** MeOH:THF:buffer 50:7:43 (Buffer was prepared from equal volumes of 22 g/L KH<sub>2</sub>PO<sub>4</sub> and 18 g/L Na<sub>2</sub>HPO<sub>4</sub>, pH 6.6 ± 0.2.)

**Flow rate:** 2

**Injection volume:** 6

**Detector:** UV 240

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**CHROMATOGRAM**

**Retention time:** 4.1

**Internal standard:** barbital (0.8), thiamylal (5.2)

**Limit of detection:** 1 µg/mL

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**OTHER SUBSTANCES**

**Extracted:** pentobarbital

**Simultaneous:** acetaminophen, acetazolamide, amobarbital, aspirin, butabarbital, cefazolin, chlorothiazide, chlorzoxazone, dicloxacillin, ethosuximide, furosemide, hydrochlorothiazide, ibuprofen, oxacillin, phenobarbital, phenylbutazone, phenytoin, salicylic acid, secobarbital, sulfamethoxazole, theophylline, ascorbic acid

**Noninterfering:** ampicillin, penicillin G, valproic acid

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**KEY WORDS**

serum

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**REFERENCE**

Kelner,M.; Bailey,D.N. Reversed-phase liquid-chromatographic simultaneous analysis for thiopental and pentobarbital in serum, *Clin.Chem.*, **1983**, *29*, 1097–1100.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 1 mL Plasma or hemolyzed (frozen and thawed) whole blood + 500 µL 2 µg/mL hexobarbital in water + 500 µL 250 mM HCl + 40 mg NaCl + 3 mL toluene, rotate, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at 50 ± 5°, reconstitute the residue in 200 µL MeCN and 200 µL 50 mM NaH<sub>2</sub>PO<sub>4</sub>, inject a 20 µL aliquot.

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**HPLC VARIABLES**

**Guard column:** 30 × 4 Perisorb RP-18 (Merck)

**Column:** 250 × 4 7 µm LiChroCart RP-18 (Merck)

**Mobile phase:** MeCN:50 mM pH 4.6 NaH<sub>2</sub>PO<sub>4</sub> 50:50

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 195

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**CHROMATOGRAM****Retention time:** 6.8**Internal standard:** hexobarbital (4.8)**Limit of quantitation:** 250 ng/mL

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**OTHER SUBSTANCES****Extracted:** methohexital**Simultaneous:** barbital, caffeine, indomethacin, pentobarbital, phenobarbital**Noninterfering:** aspirin, salicylic acid

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**KEY WORDS**plasma; whole blood; pharmacokinetics

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**REFERENCE**Bjorkman,S.; Idvall,J. A high-performance liquid chromatographic method for methohexital and thiopental in plasma or whole blood, *J.Chromatogr.*, **1984**, *307*, 481–487.

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**SAMPLE****Matrix:** blood**Sample preparation:** Whole blood, serum. 500  $\mu$ L Blood or serum + 500  $\mu$ L buffer + 200  $\mu$ g phenolphthalein + 5 mL dichloromethane, rotate, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 300  $\mu$ L MeOH, inject a 20  $\mu$ L aliquot. (Prepare buffer by mixing 500 mM  $\text{KH}_2\text{PO}_4$  and 500 mM  $\text{Na}_2\text{HPO}_4$  to obtain a pH of 5.5.)

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**HPLC VARIABLES****Column:** 250  $\times$  4.6 10  $\mu$ m RP-8**Mobile phase:** MeOH:water 60:40**Flow rate:** 2**Injection volume:** 20**Detector:** UV 290

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**KEY WORDS**whole blood; serum

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**REFERENCE**Levine,B.S.; Blanke,R.V.; Valentour,J.C. Postmortem stability of barbiturates in blood and tissues, *J.Forensic Sci.*, **1984**, *29*, 131–138.

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**SAMPLE****Matrix:** blood**Sample preparation:** 50  $\mu$ L 25  $\mu$ g/mL 5-Ethyl-5-p-tolylbarbituric acid in MeOH added to 150  $\times$  10 mm glass centrifuge tube and blow dry under a stream of nitrogen, add 500  $\mu$ L plasma, add 5 mL dichloromethane, mix on rotary mixer for 5 min, centrifuge. Remove organic layer, evaporate to dryness under nitrogen, take up in 500  $\mu$ L mobile phase, inject a 40  $\mu$ L aliquot.

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**HPLC VARIABLES****Guard column:** 50  $\times$  4  $\mu$ Bondapak C18 Corasil B**Column:** 300  $\times$  4 5  $\mu$ m  $\mu$ Bondapak C18**Mobile phase:** MeOH:10 mM potassium phosphate adjusted to pH 4.40  $\pm$  0.05 with 150 mM phosphoric acid 50:50**Flow rate:** 1.7**Injection volume:** 40**Detector:** UV 284

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**CHROMATOGRAM****Retention time:** 8.90**Internal standard:** 5-ethyl-5-p-tolylbarbituric acid (UV 212) (4.62)**Limit of detection:** 500 ng/mL

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**OTHER SUBSTANCES****Extracted:** pentobarbital (UV 212)

**Simultaneous:** acetaminophen, amobarbital, barbital, butalbital, butabarbital, caffeine, carbamazepine, phenacetin, phenobarbital, phenytoin, secobarbital, theobromine, theophylline, vinbarbital

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**KEY WORDS**

plasma

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**REFERENCE**

Houdret,N.; Lhermitte,M.; Lalau,G.; Izydorczak,J.; Roussel,P. Determination of thiopental and pentobarbital in plasma using high-performance liquid chromatography, *J.Chromatogr.*, **1985**, 343, 437–442.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Condition a 100 mg Bond-Elut C8 SPE cartridge with 2 volumes of MeOH, 2 volumes of water, and 1 volume of 100 mM pH 5.59 Sørensen's phosphate buffer. 200  $\mu$ L Plasma + 10  $\mu$ L 1 mg/mL sodium secobarbital in EtOH, add to the SPE cartridge, wash with 1 volume of water, elute with 250  $\mu$ L MeOH. Evaporate the eluate to dryness under vacuum, reconstitute in 50  $\mu$ L MeOH, inject an aliquot.

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**HPLC VARIABLES**

**Guard column:** 10  $\mu$ m Guard-Pak C18 (Waters)

**Column:** 100  $\times$  8 10  $\mu$ m Radial-Pak C8 (Waters)

**Mobile phase:** MeOH:THF:100 mM pH 7.72 Sørensen's phosphate buffer 28:16:52 containing 5 ng/mL sodium thiopental

**Flow rate:** 2.5

**Injection volume:** 50

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 8.54

**Internal standard:** secobarbital (6.38)

**Limit of quantitation:** 250 ng/mL

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**OTHER SUBSTANCES**

**Extracted:** methohexital, pentobarbital

**Noninterfering:** ketamine

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**KEY WORDS**

plasma; dog; pharmacokinetics; SPE

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**REFERENCE**

Avram,M.J.; Krecjcie,T.C. Determination of sodium pentobarbital and either sodium methohexital or sodium thiopental in plasma by high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1987**, 414, 484–491.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 100  $\mu$ L Serum + 100  $\mu$ L buffer + 1.5 mL IS in 5% isopropanol in chloroform, vortex for 30 s, centrifuge. Remove the organic layer and evaporate it to dryness under a stream of air at room temperature, reconstitute the residue in 100  $\mu$ L mobile phase, inject a 6–10  $\mu$ L aliquot. (Buffer was 13.6 g  $\text{KH}_2\text{PO}_4$  in 90 mL water, pH adjusted to 6.8 with about 3 mL 10 M NaOH, made up to 100 mL.)

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**HPLC VARIABLES**

**Guard column:** 20  $\times$  4.6 Supelguard LC-1 (Supelco)

**Column:** 250  $\times$  4.6 5  $\mu$ m Supelcosil LC-1 (Supelco)

**Mobile phase:** MeOH:MeCN:buffer 17.5:17.5:65 (Buffer was 2.72 g  $\text{KH}_2\text{PO}_4$  in 1.9 L water, pH adjusted to 6.3 with about 2 mL 1 M NaOH, made up to 2 L.)

**Flow rate:** 2

**Injection volume:** 6–10

**Detector:** UV 285

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**CHROMATOGRAM****Retention time:** 7.87**Internal standard:** 3-isobutyl-1-methylxanthine (3.15)

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**OTHER SUBSTANCES****Extracted:** acetaminophen, amobarbital, barbital, caffeine, carbamazepine, chloramphenicol, ethosuximide, mephobarbital, methsuximide, pentobarbital, phenobarbital, phenytoin, primidone, secobarbital, theophylline**Also analyzed:** acetanilide, N-acetylcysteine, N-acetylprocainamide, ampicillin, aspirin, butabarbital, butalbital, chlorpropamide, cimetidine, codeine, cyheptamide, diazoxide, diflunisal, diphylline, disopyramide, ethchlorvynol, gentisic acid, glutethimide, heptabarbital, hexobarbital, ibuprofen, indomethacin, ketoprofen, mefenamic acid, mephentyoin, methaqualone, methsuximide, methyl salicylate, methypyrrolon, morphine, naproxen, nirvanol, oxphenylbutazone, phenacetin, phensuximide, phenylbutazone, procainamide, salicylamide, salicylic acid, sulfamethoxazole, sulindac, tolmetin, trimethoprim, vancomycin**Noninterfering:** amikacin, gentamicin, meprobamate, netilmicin, quinidine, tetracycline, tobramycin, valproic acid

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**KEY WORDS**

serum

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**REFERENCE**Meatherall,R.; Ford,D. Isocratic liquid chromatographic determination of theophylline, acetaminophen, chloramphenicol, caffeine, anticonvulsants, and barbiturates in serum, *Ther.Drug Monit.*, **1988**, *10*, 101–115.

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**SAMPLE****Matrix:** blood**Sample preparation:** 100  $\mu$ L Plasma + 100  $\mu$ L MeCN, vortex for 10 s, let stand for 10 min, vortex for 10 s, centrifuge at 12000 g for 2 min, inject a 20  $\mu$ L aliquot of the supernatant.

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**HPLC VARIABLES****Column:** 150  $\times$  6 5  $\mu$ m Shim-pack CLC-ODS (Shimadzu)**Mobile phase:** MeCN:water 55:45**Flow rate:** 1.2**Injection volume:** 20**Detector:** UV 288

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**CHROMATOGRAM****Retention time:** 4.5

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**OTHER SUBSTANCES****Extracted:** thiamylal**Noninterfering:** acetaminophen, allopurinol, amikacin, amobarbital, amphotericin B, ampicillin, aspirin, barbital, caffeine, carbenicillin, chloramphenicol, chlorpromazine, cimetidine, cisplatin, cyclophosphamide, cyclosporin A, cytarabine, dactinomycin, doxorubicin, droperidol, ethosuximide, 5-fluorocytosine, 5-fluorouracil, furosemide, gentamicin, hexobarbital, ketamine, ketoconazole, 6-mercaptopurine, metharbital, methotrexate, miconazole, mizoribine, pentobarbital, phenobarbital, procainamide, secobarbital, tegafur, vancomycin

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**KEY WORDS**

plasma

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**REFERENCE**Hosotsubo,H.; Takeda,K.; Hosotsubo,K.; Yoshiya,I. Measurement of thiamylal in human plasma using reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *487*, 204–209.

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**SAMPLE****Matrix:** blood**Sample preparation:** Vigorously shake equal volumes of plasma and MeCN, centrifuge at 10000 g for 3 min, inject a 20  $\mu$ L aliquot of the supernatant.

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**HPLC VARIABLES**

**Column:** 110 × 4.6 PartiSphere C8 (Whatman)

**Mobile phase:** MeCN:120 mM pH 6.2 phosphate buffer 50:50

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 270 following post-column reaction. The column effluent flowed through a 6 m × 0.25 mm ID crocheted PTFE coil irradiated with a Sylvania G8-T5 lamp at 254 nm to the detector.

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**CHROMATOGRAM**

**Retention time:** 4.25

**Limit of detection:** 200 ng/mL

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**OTHER SUBSTANCES**

**Extracted:** pentobarbital

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**KEY WORDS**

post-column reaction; post-column photochemical derivatization; plasma

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**REFERENCE**

Schmid,R.W.; Wolf,C. Simultaneous determination of thiopental and its metabolite, pentobarbital, in blood by high-performance liquid chromatography and post-column photochemical reaction, *J.Pharm.Biomed.Anal.*, 1989, 7, 1749–1755.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 200-500 µL Whole blood + 1 mL 100 mM pH 7.5 phosphate buffer, vortex for 1 min, add 7 mL n-hexane:diethyl ether 50:50, add 50 µL 100 µg/mL secobarbital in EtOH: water 75:25, shake for 15 min, centrifuge at 4° at 2000 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 µL mobile phase, vortex, inject a 5-20 µL aliquot.

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**HPLC VARIABLES**

**Column:** 100 × 3 5 µm Nucleosil C18

**Mobile phase:** MeCN:water 32:68

**Flow rate:** 0.3

**Injection volume:** 5-20

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 13

**Internal standard:** secobarbital (9)

**Limit of detection:** 10 ng/mL

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**OTHER SUBSTANCES**

**Extracted:** pentobarbital

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**KEY WORDS**

whole blood; pharmacokinetics

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**REFERENCE**

Celardo,A.; Bonati,M. Determination of thiopental measured in human blood by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, 1990, 527, 220–225.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 2 mL Plasma + 1.2 mL buffer, mix gently, add 10 mL toluene, shake for 5 min (break any emulsion with sonication), centrifuge at 1000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 100 µL MeOH, inject a 50 µL aliquot. (Buffer was 100 mM acetic acid:100 mM sodium acetate 76:24, pH 4.5.)

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**HPLC VARIABLES****Column:** 300 × 4.6 5 µm Nucleosil C18**Mobile phase:** MeCN:MeOH:buffer:150 mM NaCl 27:27:36:10 (Buffer was 9.00 g KH<sub>2</sub>PO<sub>4</sub> and 140 mg Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O in 1 L water, pH 5.05.)**Flow rate:** 2.5**Injection volume:** 20**Detector:** UV 340

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**CHROMATOGRAM****Retention time:** 4**Internal standard:** thiopental

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**OTHER SUBSTANCES****Extracted:** progabide

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**KEY WORDS**plasma; thiopental is IS

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**REFERENCE**Decourt,J.P.; Mura,P.; Papet,Y.; Piriou,A.; Reiss,D. Simultaneous determination of progabide and its acid metabolite by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1990**, 527, 214–219.

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**SAMPLE****Matrix:** blood**Sample preparation:** 500 µL Serum or plasma + 100 µL 20 µg/mL thiamylal in MeOH + 1 mL 70 mM pH 6.4 phosphate buffer + 5 mL n-pentane, shake vigorously for 10 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 µL mobile phase, vortex for 1 min, inject a 5 µL aliquot.

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**HPLC VARIABLES****Column:** Nucleosil 5 C8**Mobile phase:** MeCN:buffer 35:65 (Buffer was 0.2 mM phosphoric acid containing 0.175 mM KH<sub>2</sub>PO<sub>4</sub>.)**Flow rate:** 1.2**Injection volume:** 5**Detector:** UV 290

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**CHROMATOGRAM****Retention time:** 10**Internal standard:** thiamylal (12)

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**KEY WORDS**serum; plasma; cow; human; comparison with capillary electrophoresis

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**REFERENCE**Meier,P.; Thormann,W. Determination of thiopental in human serum and plasma by high-performance capillary electrophoresis-micellar electrokinetic chromatography, *J.Chromatogr.*, **1991**, 559, 505–513.

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**SAMPLE****Matrix:** blood**Sample preparation:** 300 µL Plasma + 20 µL 500 µg/mL phenylbutazone in 2 mM NaOH + 1.1 mL ether:n-hexane 20:80 + 20 µL 3 M phosphoric acid, vortex at 1200 rpm for 1 min, centrifuge at 2000 g for 5 min, freeze in dry ice for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure (16 mbar) at 40° for 10 min, reconstitute the residue in 50 µL 400 µM NaOH, inject a 2–40 µL aliquot.

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**HPLC VARIABLES****Guard column:** 10 × 3 AGP (ChromTech)**Column:** 100 × 4 AGP-CSP (ChromTech)**Mobile phase:** Isopropanol:100 mM pH 6.2 phosphate buffer 4.5:95.5



**Flow rate:** 0.9  
**Injection volume:** 2-40  
**Detector:** UV 287

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#### CHROMATOGRAM

**Retention time:** 7.1 (R(+)), 10.8 (S(-))  
**Internal standard:** phenylbutazone (15.7)  
**Limit of quantitation:** 6 ng/mL (S(-)), 5 ng/mL (R(+))

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#### OTHER SUBSTANCES

**Extracted:** pentobarbital (UV 220)

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#### KEY WORDS

sheep; plasma; chiral; pharmacokinetics

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#### REFERENCE

Huang,J.L.; Mather,L.E.; Duke,C.C. High-performance liquid chromatographic determination of thiopentone enantiomers in sheep plasma, *J.Chromatogr.B*, **1995**, 673, 245-250.

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#### SAMPLE

**Matrix:** blood

**Sample preparation:** Mix serum with an equal volume of 1 M pH 5.0 phosphate buffer, add IS, add 3 mL ethyl acetate, rotate for 10 min, centrifuge at 3500 rpm for 5 min. Remove 2 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 200  $\mu$ L mobile phase, inject a 10-20  $\mu$ L aliquot.

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#### HPLC VARIABLES

**Column:** 150  $\times$  6 Shim-pack CLC-ODS (Shimadzu)

**Mobile phase:** MeOH:10 mM pH 5.0 sodium phosphate buffer 55:45

**Flow rate:** 1

**Injection volume:** 10-20

**Detector:** UV

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#### CHROMATOGRAM

**Internal standard:** 5-(p-methylphenyl)-5-phenylhydantoin

**Limit of quantitation:** 1  $\mu$ g/mL

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#### OTHER SUBSTANCES

**Also analyzed:** pentobarbital

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#### KEY WORDS

serum; rat; pharmacokinetics

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#### REFERENCE

Nakashima,E.; Matsushita,R.; Ohshima,T.; Tsuji,A.; Ichimura,F. Quantitative relationship between structure and peritoneal membrane transport based on physiological pharmacokinetic concepts for acidic drugs, *Drug Metab.Dispos.*, **1995**, 23, 1220-1224.

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#### SAMPLE

**Matrix:** blood

**Sample preparation:** 2 mL Plasma + 2 mL 34 mg/mL pH 5.5  $\text{KH}_2\text{PO}_4$  + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under reduced pressure at 45°, reconstitute the residue in 100  $\mu$ L mobile phase, centrifuge at 10000 g for 5 min, inject a 50  $\mu$ L aliquot of the supernatant.

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#### HPLC VARIABLES

**Column:** 300  $\times$  3.9  $\mu$ m NovaPak C18

**Mobile phase:** MeOH:THF:0.68 mg/mL pH 2.6  $\text{KH}_2\text{PO}_4$  65:5:30

**Column temperature:** 30

Flow rate: 0.8  
Injection volume: 50  
Detector: UV 293

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**CHROMATOGRAM**

Retention time: 6.0  
Internal standard: thiopental

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**OTHER SUBSTANCES**

Extracted: dapsone

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**KEY WORDS**

plasma; thiopental is IS

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**REFERENCE**

Tracqui, A.; Gutbub, A.M.; Kintz, P.; Mangin, P. A case of acute dapsone poisoning: Toxicological data and review of the literature, *J. Anal. Toxicol.*, **1995**, *19*, 229–235.

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**SAMPLE**

Matrix: blood

**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

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**HPLC VARIABLES**

Column: 300 × 3.9 4 µm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH<sub>2</sub>PO<sub>4</sub> adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 287

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**CHROMATOGRAM**

Retention time: 5.26

Limit of detection: <120 ng/mL

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**KEY WORDS**

whole blood; plasma; interferences may occur—compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfapyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vandesine; mexiletine; dipyrindamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazo-

lam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proganil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

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## REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254-262.

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## SAMPLE

**Matrix:** blood

**Sample preparation:** 250  $\mu$ L Plasma + 50  $\mu$ L 200  $\mu$ g/mL ketamine in MeOH + 1 mL 10 mM pH 6.0  $\text{KH}_2\text{PO}_4$  buffer + 3 mL pentane, vortex for 20 s, centrifuge at 4000 g for 10 min, freeze in dry ice. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 120  $\mu$ L mobile phase, vortex for 10 s, inject a 10-100  $\mu$ L aliquot.

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## HPLC VARIABLES

**Guard column:** 10  $\times$  3 Chromtec chiral AGP (J.T. Baker)

**Column:** 100  $\times$  4 Chromtec chiral AGP (J.T. Baker)

**Mobile phase:** MeOH:isopropanol:20 mM phosphate buffer 1.5:5:93.5, pH 5.0 (At the end of each day wash with isopropanol:water 10:90 at 0.1 mL/min overnight.)

**Flow rate:** 0.9

**Injection volume:** 10-100

**Detector:** UV 280

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## CHROMATOGRAM

**Retention time:** 4.84 (R-+), 6.18 (S-)

**Internal standard:** ketamine (1.84)

**Limit of quantitation:** 10 ng/mL

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## KEY WORDS

plasma; chiral; pharmacokinetics

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## REFERENCE

Jones,D.J.; Nguyen,K.T.; McLeish,M.J.; Crankshaw,D.P.; Morgan,D.J. Determination of (R)-(+)- and (S)-(-)-isomers of thiopentone in plasma by chiral high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *675*, 174-179.

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## SAMPLE

**Matrix:** blood, CSF, gastric contents, urine

**Sample preparation:** 200  $\mu$ L Serum, urine, CSF, or gastric fluid + 300  $\mu$ L reagent. Flush column A to waste with 500  $\mu$ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500  $\mu$ L 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

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## HPLC VARIABLES

**Column:** A 40  $\mu$ m preparative grade C18 (Analytichem); B 75  $\times$  2.1 pellicular C18 (Whatman) + 250  $\times$  4.6 5  $\mu$ m C8 end-capped (Whatman)

**Mobile phase:** Gradient. A was 50 mM pH 4.5  $\text{KH}_2\text{PO}_4$ . B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.

**Column temperature:** 50**Flow rate:** 1.5**Detector:** UV 220

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**CHROMATOGRAM****Retention time:** 14.1**Internal standard:** heptanophenone (19)

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**OTHER SUBSTANCES**

**Extracted:** acetaminophen, allobarbitol, azinphos, barbitol, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thioridazine, trimethoprim

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**KEY WORDS**

serum; column-switching

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**REFERENCE**

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J. Chromatogr.*, **1993**, 612, 191–198.

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**SAMPLE****Matrix:** blood, tissue

**Sample preparation:** Tissue. Homogenize tissue with 2 volumes of water. 1 mL Homogenate + 300  $\mu$ L 1 mg/mL phenolphthalein in MeOH + 1 mL buffer + 10 mL dichloromethane, rotate, centrifuge. Remove the organic layer and add it to 3 mL 100 mM NaOH, rotate, centrifuge. Remove the aqueous layer and acidify it with 1 mL 1 M HCl, extract with 10 mL dichloromethane. Remove the organic layer and evaporate it to dryness at 40°, reconstitute the residue in 300  $\mu$ L MeOH, inject a 20  $\mu$ L aliquot. Blood. 500  $\mu$ L Whole blood + 500  $\mu$ L buffer + 200  $\mu$ L 1 mg/mL phenolphthalein in MeOH + 5 mL dichloromethane, rotate, centrifuge. Remove the organic layer and evaporate it to dryness at 40°, reconstitute the residue in 300  $\mu$ L MeOH, inject a 20  $\mu$ L aliquot. (Buffer was prepared by mixing 500 mM  $\text{Na}_2\text{HPO}_4$  with 500 mM  $\text{KH}_2\text{PO}_4$  to pH 5.5.)

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**HPLC VARIABLES****Column:** 250  $\times$  4.6 10  $\mu$ m RP-8 (Hewlett-Packard) or 260  $\times$  4.6 10  $\mu$ m Spherisorb C18**Mobile phase:** MeOH:water 60:40**Flow rate:** 2**Injection volume:** 20**Detector:** UV 290

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**CHROMATOGRAM****Retention time:** 5.97**Internal standard:** phenolphthalein (2.96)**Limit of quantitation:** 1  $\mu$ g/mL

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**OTHER SUBSTANCES****Simultaneous:** carbamazepine, pentobarbital (UV 210)**Noninterfering:** amobarbital, butobarbital, glutethimide, meprobamate, methaqualone, methyprylon, phenobarbital, phenytoin, secobarbital

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**KEY WORDS**

whole blood

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**REFERENCE**

Levine,B.; Blanke,R.; Valentour,J. Liquid chromatographic analysis of thiopental in blood and tissues, *J.Anal.Toxicol.*, **1983**, 7, 207–208.

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**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50  $\mu$ L MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood)  $\mu$ L aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

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**HPLC VARIABLES**

**Guard column:** 20 mm long Symmetry C18

**Column:** 250  $\times$  4.6 5  $\mu$ m Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10-30

**Detector:** UV 285.5

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**CHROMATOGRAM**

**Retention time:** 19.202

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**KEY WORDS**

whole blood

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**REFERENCE**

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, 763, 149-163.

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**SAMPLE**

**Matrix:** formulations

**Sample preparation:** Dilute equal volume 10 mg/mL propofol and 25 mg/mL thiopental injections 1:200 with water, inject a 20  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 150  $\times$  4.6 5  $\mu$ m Zorbax SB

**Mobile phase:** MeCN:buffer 45:55 (Buffer was 10 mM  $\text{KH}_2\text{PO}_4$ , adjusted to pH 4.0 with 10% phosphoric acid.)

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 235

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**CHROMATOGRAM**

**Retention time:** 4.9

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**OTHER SUBSTANCES**

**Simultaneous:** propofol

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**KEY WORDS**

stability-indicating; injections

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**REFERENCE**

Chernin,E.L.; Stewart,J.T.; Smiler,B. Stability of thiopental sodium and propofol in polypropylene syringes at 23 and 4°C, *Am.J.Health-Syst.Pharm.*, **1996**, 53, 1576-1579.

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**SAMPLE****Matrix:** formulations**Sample preparation:** Dilute with saline, inject a 10  $\mu$ L aliquot.

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**HPLC VARIABLES****Column:** 250  $\times$  4.6 Lichrosorb 10 RP 8**Mobile phase:** MeOH:THF:water 50:5:50**Flow rate:** 3**Injection volume:** 10**Detector:** UV 254

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**CHROMATOGRAM****Retention time:** 11.2

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**OTHER SUBSTANCES****Simultaneous:** diazepam, lorazepam

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**KEY WORDS**

injections; saline

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**REFERENCE**Martens,H.J.; de Goede,P.N.; van Loenen,A.C. Sorption of various drugs in polyvinyl chloride, glass, and polyethylene-lined infusion containers, *Am.J.Hosp.Pharm.*, **1990**, 47, 369–373.

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**SAMPLE****Matrix:** solutions**Sample preparation:** Add 380  $\mu$ g propofol and 1.03 mg thiopental sodium to 0.9% sodium chloride, shake vigorously for 2 min, make up to 10 mL with 0.9% sodium chloride, inject a 20  $\mu$ L aliquot.

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**HPLC VARIABLES****Column:** 150  $\times$  4.6 5  $\mu$ m Zorbax SB phenyl**Mobile phase:** MeCN:buffer 45:55 (Buffer was 10 mM  $\text{KH}_2\text{PO}_4$  adjusted to pH 4.0 with 10% phosphoric acid.)**Flow rate:** 1**Injection volume:** 20**Detector:** UV 235

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**CHROMATOGRAM****Retention time:** 4.9**Limit of detection:** 317 ng/mL

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**OTHER SUBSTANCES****Simultaneous:** propofol

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**REFERENCE**King,D.T.; Stewart,J.T.; Venkateshwaran,T.G. HPLC determination of propofol-thiopental sodium and propofol-ondansetron mixtures, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, 19, 2285–2294.

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**SAMPLE****Matrix:** solutions

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**HPLC VARIABLES****Guard column:** 30  $\times$  3.2 7  $\mu$ m SI 100 ODS (not commercially available)**Column:** 150  $\times$  3.2 7  $\mu$ m SI 100 ODS (not commercially available)**Mobile phase:** MeCN:buffer 31.2:68.8 (Buffer was 6.66 g  $\text{KH}_2\text{PO}_4$  and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)**Flow rate:** 0.5–1**Detector:** UV 232, 282

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**CHROMATOGRAM****Retention time:** 10.4**Internal standard:** 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

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**OTHER SUBSTANCES**

**Also analyzed:** aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, tetracaine, triamterene, verapamil, zolpidem, zopiclone

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**REFERENCE**

Below, E.; Burrmann, M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J. Liq. Chromatogr.*, **1994**, 17, 4131–4144.

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**SAMPLE****Matrix:** solutions**Sample preparation:** Dissolve in mobile phase to a concentration of 50 µg/mL.

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**HPLC VARIABLES****Column:** 250 × 4 β-cyclodextrin polymer-coated silica (Chromatographia 1993, 36, 373)**Mobile phase:** MeOH:water 50:50**Flow rate:** 0.6**Injection volume:** 20**Detector:** UV 240

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**CHROMATOGRAM****Retention time:** k' 4.07

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**OTHER SUBSTANCES**

**Simultaneous:** aprobarbital, pentobarbital, amobarbital, butabarbital, butalbital, phenobarbital, secobarbital

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**REFERENCE**

Forgács, E.; Cserhádi, T. Retention behaviour of barbituric acid derivatives on a β-cyclodextrin polymer-coated silicon column, *J. Chromatogr. A*, **1994**, 668, 395–402.

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**SAMPLE****Matrix:** solutions

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**HPLC VARIABLES****Column:** 250 × 4.6 5 µm Supelcosil LC-DP (A) or 250 × 4 5 µm LiChrospher 100 RP-8 (B)

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6**Injection volume:** 25**Detector:** UV 229

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**CHROMATOGRAM****Retention time:** 7.04 (A), 7.33 (B)

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**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordi-azepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphen-oxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide,

ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazinol, mefenamic acid, meperidine, mephénytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thioridazine, thiothixene, timolol, tocinamide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, trifluorpromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

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**KEY WORDS**

details of plasma extraction

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**REFERENCE**

Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J. Chromatogr. A*, **1995**, 692, 103–119.

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**SAMPLE**

**Matrix:** tissue

**Sample preparation:** Homogenize (Brinkman Polytron Model PT 10/35) 0.5–1.5 g tissue in 3 mL 70 mM pH 7.4 Sorensen's phosphate buffer with 10 s bursts while chilling with ice. 0.2–1 mL Plasma or tissue homogenate + 1 mL 70 mM pH 6.3 Sorensen's phosphate buffer + 50  $\mu$ L 20  $\mu$ g/mL thiamylal in MeOH:water 50:50 + 5 mL pentane, shake at 40 cycles/min for 10 min, centrifuge, freeze in dry ice/acetone. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200  $\mu$ L MeCN:water 25:75, inject a 100  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 10  $\mu$ m C18 (Alltech)

**Mobile phase:** MeCN:4 mM potassium phosphate buffer 50:50, pH 4.0

**Flow rate:** 1.2

**Injection volume:** 100

**Detector:** UV 290

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**CHROMATOGRAM**

**Retention time:** 5.5

**Internal standard:** thiamylal (6.5)

**Limit of detection:** 10 ng/mL

**Limit of quantitation:** 50 ng/mL

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**OTHER SUBSTANCES**

**Simultaneous:** amobarbital, heptabarbital, hexobarbital, methohexital, pentobarbital, phenobarbital, secobarbital

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**KEY WORDS**

rat; plasma; brain; fat; heart; intestine; kidney; liver; lung; muscle; pancreas; plasma; spleen; testes



## REFERENCE

Ebling, W.F.; Mills-Williams, L.; Harapat, S.R.; Stanski, D.R. High-performance liquid chromatographic method for determining thiopental concentrations in twelve rat tissues: application to physiologic modeling of disposition of barbiturate, *J. Chromatogr.*, **1989**, *490*, 339–353.

# Thiopropazate

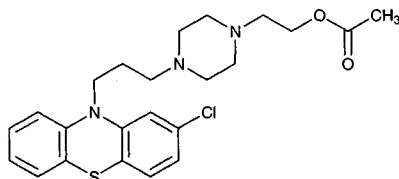
**Molecular formula:**  $C_{23}H_{28}ClN_3O_2S$

**Molecular weight:** 446.01

**CAS Registry No.:** 84-06-0, 146-28-1 (2.HCl), 104999-18-0 (dimalate)

**Merck Index:** 9493

**Lednicer No.:** 1 383



## SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

## HPLC VARIABLES

**Column:** 125 × 4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

## CHROMATOGRAM

**Retention time:** 1.8

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocaine, benzocamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimetazoline, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, naltrexone, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenylglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxypromazine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, pro-

heptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenylamine, theophylline, thiethylperazine, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelethamine, triprolidine, tryptamine, verapamil, xylometazoline

## REFERENCE

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, *323*, 191–225.

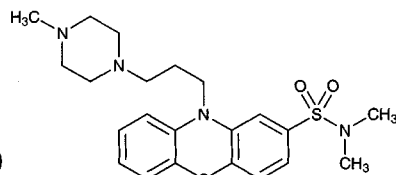
# Thioproperazine

**Molecular formula:**  $C_{22}H_{30}N_4O_2S_2$

**Molecular weight:** 446.64

**CAS Registry No.:** 316-81-4, 2347-80-0 (dimethanesulfonate)

**Merck Index:** 9494



## SAMPLE

**Matrix:** blood

**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100  $\mu$ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50  $\mu$ L aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

## HPLC VARIABLES

**Column:** 300  $\times$  3.9 4  $\mu$ m NovaPack C18

**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic))  $KH_2PO_4$  adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

**Column temperature:** 30

**Flow rate:** 0.8

**Injection volume:** 50

**Detector:** UV 266

## CHROMATOGRAM

**Retention time:** 7.47

**Limit of detection:** <120 ng/mL

## KEY WORDS

whole blood; plasma; interferences may occur—compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; tolaxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procabazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; almino-

profen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vandesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phenacyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loperamide; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacine; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenopropfen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thiopropazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozone; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

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## REFERENCE

Tracqui, A.; Kintz, P.; Mangin, P. Systematic toxicological analysis using HPLC/DAD, *J. Forensic Sci.*, **1995**, *40*, 254–262.

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## SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50  $\mu$ L MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood)  $\mu$ L aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200–350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

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## HPLC VARIABLES

**Guard column:** 20 mm long Symmetry C18

**Column:** 250  $\times$  4.6 5  $\mu$ m Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10–30

**Detector:** UV 265.3

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## CHROMATOGRAM

**Retention time:** 15.212

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## KEY WORDS

whole blood

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## REFERENCE

Gaillard, Y.; Pépin, G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J. Chromatogr. A*, **1997**, *763*, 149–163.

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## SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a 10  $\mu$ g/mL solution in MeOH, inject a 20  $\mu$ L aliquot.

**HPLC VARIABLES****Column:** 125 × 4.9 Spherisorb S5W silica**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7**Flow rate:** 2**Injection volume:** 20**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V**CHROMATOGRAM****Retention time:** 4.5**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranlycpromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleannamine, triprolidine, tryptamine, verapamil, xylometazoline

**REFERENCE**

Jane, I.; McKinnon, A.; Flanagan, R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, *323*, 191–225.

# Thioridazine

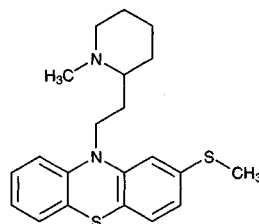
**Molecular formula:**  $C_{21}H_{26}N_2S_2$

**Molecular weight:** 370.58

**CAS Registry No.:** 50-52-2, 130-61-0 (HCl)

**Merck Index:** 9497

**Lednicer No.:** 1 389



## SAMPLE

**Matrix:** blood

**Sample preparation:** 1-5 mL Plasma + 1 mL 1 M NaOH + hexanes, extract for 30 min, centrifuge. Remove a 9 mL aliquot of the organic phase and evaporate it to dryness at 30° under a stream of nitrogen. Dissolve the residue in 100 µL mobile phase, inject a 50 µL aliquot.

## HPLC VARIABLES

**Column:** 10 µm Micropak CN (Varian)

**Mobile phase:** MeCN:5 mM ammonium acetate 90:10

**Flow rate:** 2.5

**Injection volume:** 50

**Detector:** UV 254

## CHROMATOGRAM

**Retention time:** 29.5

**Limit of detection:** 10 ng/mL

## OTHER SUBSTANCES

**Simultaneous:** acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, fluphenazine, promethazine, haloperidol, imipramine, mesoridazine, nortriptyline, orphenadrine, piperacetazine, promazine, chlorpromazine, thiothixene, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine, metabolites

## KEY WORDS

plasma

## REFERENCE

Curry, S.H.; Brown, E.A.; Hu, O.Y.-P.; Perrin, J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J. Chromatogr.*, **1982**, 231, 361-376.

## SAMPLE

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 2 mL water + 2 mL 2 M NaOH, mix well, add 10 mL heptane:isoamyl alcohol 99:1, shake slowly on a reciprocating shaker for 15 min, centrifuge at 5-10° at 1207 g for 5 min. Remove 8.5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 60°, reconstitute the residue in 250 µL MeCN:water 60:40, inject a 50 µL aliquot.

## HPLC VARIABLES

**Column:** 250 × 4.6 5 µm LC-PCN (cyano) (Supelco)

**Mobile phase:** MeCN:20 mM pH 4.5  $KH_2PO_4$  60:40

**Column temperature:** 40

**Flow rate:** 2

**Injection volume:** 50

**Detector:** UV 229 or E, IBM Model 230, Model 3892 glassy carbon electrode, 1000 mV vs saturated calomel electrode

## CHROMATOGRAM

**Retention time:** 8.5

**Internal standard:** thioridazine

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**OTHER SUBSTANCES**

**Extracted:** chlorprothixene

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**KEY WORDS**

plasma; thioridazine is IS

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**REFERENCE**

Brooks, M.A.; DiDonato, G.; Blumenthal, H.P. Determination of chlorprothixene and its sulfoxide metabolite in plasma by high-performance liquid chromatography with ultraviolet and amperometric detection, *J. Chromatogr.*, **1985**, 337, 351–362.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Condition a Varian AASP C18 SPE cartridge with 1 mL MeOH and 1 mL water. Mix 500  $\mu$ L serum with 500  $\mu$ L 200 mM phosphoric acid (pH was ca. 2.3), let stand for 5 min, add 400  $\mu$ L to the SPE cartridge, wash with 1 mL water, wash with 1 mL MeCN:water 1:1, elute cartridge with mobile phase for 30 s directly onto the analytical column.

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**HPLC VARIABLES**

**Column:** 250  $\times$  4.5  $\mu$ m Spherisorb ODS-2 Suprapac

**Mobile phase:** MeCN:MeOH:buffer 25:50:25, pH was 4.1. (Buffer was 4.5 mL 85% orthophosphoric acid and 4.5 mL triethylamine in 1 L water.)

**Flow rate:** 1

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 10.95

**Limit of detection:** about 0.2  $\mu$ M

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**OTHER SUBSTANCES**

**Simultaneous:** metabolites, clonazepam, nitrazepam, flunitrazepam, lorazepam, oxazepam, alprazolam, haloperidol, diazepam, zuclopenthixol, protriptyline, nortriptyline, maprotiline, promethazine, imipramine, amitriptyline, levomepromazine, trimipramine, chlorpromazine, clomipramine, perphenazine, fluphenazine, prochlorperazine

**Noninterfering:** carbamazepine, phenytoin

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**KEY WORDS**

serum; SPE

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**REFERENCE**

Svensson, C.; Nyberg, G.; Soomägi, M.; Mårtensson, E. Determination of the serum concentrations of thioridazine and its main metabolites using a solid-phase extraction technique and high-performance liquid chromatography, *J. Chromatogr.*, **1990**, 529, 229–236.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 1 mL Serum + 25  $\mu$ L 25  $\mu$ g/mL promethazine in MeOH + 100  $\mu$ L 2 M NaOH + 4 mL hexane:ethyl acetate 1:1, rotate at 60 rpm for 5 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness at 40° under a stream of air, reconstitute with 250  $\mu$ L mobile phase A, vortex for 20 s, inject a 120  $\mu$ L aliquot onto column A with mobile phase A. Collect the effluent corresponding to the thioridazine peak, add 25  $\mu$ L 25  $\mu$ g/mL thiothixene in MeOH, vortex for 20 s, evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 250  $\mu$ L mobile phase B, inject a 120  $\mu$ L aliquot onto column B with mobile phase B.

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**HPLC VARIABLES**

**Column:** A 15  $\times$  3.2 Alltech RP-18 guard column + 250  $\times$  4.6 5  $\mu$ m Supelcosil LC18-DB; B 250  $\times$  4.6 5  $\mu$ m Spherisorb Chiral 1 (phenylmethylurea)

**Mobile phase:** A MeOH:MeCN:water:1 M ammonium hydroxide 500:350:150:3, adjusted to pH 6.7–8.0 with 6 M HCl; B Hexane:dichloromethane:MeOH:1 M ammonium acetate in MeOH 450:450:100:0.075

**Flow rate:** A 1; B 1  
**Injection volume:** 120  
**Detector:** A UV 263; B UV 263

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#### CHROMATOGRAM

**Retention time:** 7.4 (A), 9 (B) (+), 10 (-)  
**Internal standard:** A promethazine (5.8); B thiothixene (4)  
**Limit of detection:** 12.5 ng/mL  
**Limit of quantitation:** 50 ng/mL

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#### OTHER SUBSTANCES

**Simultaneous:** desipramine, nortriptyline, amitriptyline, imipramine, clozapine, maprotiline, chlordiazepoxide, thiothixene, mesoridazine, sulforidazine, fluoxetine  
**Noninterfering:** flurazepam, methylphenidate, ephedrine  
**Interfering:** chlorpromazine

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#### KEY WORDS

serum; chiral

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#### REFERENCE

Jortani, S.A.; Poklis, A. Determination of thioridazine enantiomers in human serum by sequential achiral and chiral high-performance liquid chromatography, *J. Anal. Toxicol.*, **1993**, 17, 374–377.

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#### SAMPLE

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 500 ng pipothiazine + 400  $\mu$ L 2 M NaOH + 4 mL diethyl ether:hexane 75:25, shake for 20 min, centrifuge at 2800 g for 5 min. Remove the organic layer and add it to 1.2 mL 100 mM HCl, shake for 15 min, centrifuge. Remove the aqueous layer and add it to 200  $\mu$ L 2 M NaOH, extract twice with 2 mL portions of diethyl ether:hexane 75:25. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 40°, reconstitute the residue in 120  $\mu$ L isooctane:dichloromethane:MeOH 83:10:7, inject a 100  $\mu$ L aliquot on to an 8 mm dia column of 5  $\mu$ m silica (Waters Z-module radial compression) protected by a guard column of the same material, and elute with isooctane:dichloromethane:MeOH 83:10:7 at 2.2 mL/min (detector F ex 262 em 458). Collect the fraction at 2.3 min containing thioridazine and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute with 125  $\mu$ L mobile phase, inject a 100  $\mu$ L aliquot.

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#### HPLC VARIABLES

**Column:** 250  $\times$  4.6 Cyclobond I 2000 ac  $\beta$ -acetylated cyclodextrin (Astec, Whippany)  
**Mobile phase:** MeCN:buffer 16:84 (Buffer was 1% triethylamine adjusted to pH 3.0 with orthophosphoric acid. Wash column with MeCN:water 90:10 at the end of each day.)  
**Flow rate:** 1.2  
**Injection volume:** 100  
**Detector:** F ex 262 em 458

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#### CHROMATOGRAM

**Retention time:** 17.9 (R), 19.7 (S)  
**Internal standard:** pipothiazine (9.4 min on achiral system)  
**Limit of quantitation:** 60 ng/mL (chiral), 15 ng/mL (achiral)

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#### KEY WORDS

plasma; chiral; normal phase; achiral

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#### REFERENCE

Eap, C.B.; Koeb, L.; Powell, K.; Baumann, P. Determination of the enantiomers of thioridazine, thioridazine 2-sulfone, and of the isomeric pairs of thioridazine 2-sulfoxide and thioridazine 5-sulfoxide in human plasma, *J. Chromatogr. B*, **1995**, 669, 271–279.

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#### SAMPLE

**Matrix:** blood

**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

#### HPLC VARIABLES

**Column:** 300 × 3.9 4 µm NovaPack C18

**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic))  $\text{KH}_2\text{PO}_4$  adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

**Column temperature:** 30

**Flow rate:** 0.8

**Injection volume:** 50

**Detector:** UV 264

#### CHROMATOGRAM

**Retention time:** 13.43

**Limit of detection:** <120 ng/mL

#### KEY WORDS

whole blood; plasma; interferences may occur—compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melfalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyrindamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiasepoxide; oxprenolol; warfarin; clorazepate; flecainide; phenacyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluoperidol; medazepam; yohemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenopropfen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; caripramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

#### REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

#### SAMPLE

**Matrix:** blood, CSF, gastric contents, urine



**Sample preparation:** 200  $\mu$ L Serum, urine, CSF, or gastric fluid + 300  $\mu$ L reagent. Flush column A to waste with 500  $\mu$ L 500 mM ammonium sulfate, inject sample onto column A, flush column A to waste with 500  $\mu$ L 500 mM ammonium sulfate, backflush the contents of column A onto column B with mobile phase, monitor the effluent from column B. (Reagent was 8.05 M guanidine HCl and 1.02 M ammonium sulfate in water.)

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#### HPLC VARIABLES

**Column:** A 40  $\mu$ m preparative grade C18 (Analytichem); B 75  $\times$  2.1 pellicular C18 (Whatman) + 250  $\times$  4.6 5  $\mu$ m C8 end-capped (Whatman)

**Mobile phase:** Gradient. A was 50 mM pH 4.5  $\text{KH}_2\text{PO}_4$ . B was MeCN:isopropanol 80:20. A:B 90:10 for 1 min, to 30:70 over 20 min.

**Column temperature:** 50

**Flow rate:** 1.5

**Detector:** UV 220

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#### CHROMATOGRAM

**Retention time:** 11.33

**Internal standard:** heptanophenone (19)

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#### OTHER SUBSTANCES

**Extracted:** acetaminophen, allobarbitol, azinphos, barbitol, brallobarbitone, bromazepam, butethal, caffeine, carbamazepine, carbaryl, cephaloridine, chloramphenicol, chlordiazepoxide, chlorothiazide, chlorvinphos, clothiapine, cocaine, coomassie blue, desipramine, diazepam, diphenhydramine, dipipanone, ethylbromphos, flufenamic acid, formothion, griseofulvin, indomethacin, lidocaine, lorazepam, malathion, medazepam, midazolam, oxazepam, paraoxon, penicillin G, pentobarbital, prazepam, propoxyphene, prothiophos, quinine, salicylic acid, secobarbital, strychnine, sulfamethoxazole, theophylline, thiopental, trimethoprim

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#### KEY WORDS

serum; column-switching

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#### REFERENCE

Kruger,P.B.; Albrecht,C.F.De V.; Jaarsveld,P.P. Use of guanidine hydrochloride and ammonium sulfate in comprehensive in-line sorption enrichment of xenobiotics in biological fluids by high-performance liquid chromatography, *J. Chromatogr.*, **1993**, 612, 191–198.

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#### SAMPLE

**Matrix:** blood, tissue

**Sample preparation:** Blood or serum. 1 mL Blood or serum + 1  $\mu$ g cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100  $\mu$ L 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30  $\mu$ L aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10  $\mu$ g cianopramine + 500  $\mu$ L 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400  $\mu$ L 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30  $\mu$ L aliquot of the aqueous layer.

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#### HPLC VARIABLES

**Guard column:** 15  $\times$  3.2 7  $\mu$ m RP-18 Newguard (Applied Biosystems)

**Column:** 100  $\times$  4.6 5  $\mu$ m Brownlee Spheri-5 RP-18

**Mobile phase:** MeCN:100 mM  $\text{NaH}_2\text{PO}_4$ :diethylamine 40:57.5:2.5

**Flow rate:** 2

**Injection volume:** 30

**Detector:** UV 220

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#### CHROMATOGRAM

**Retention time:** 77.76

**Internal standard:** cianopramine (8.93)

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**OTHER SUBSTANCES**

**Simultaneous:** amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, mesoridazine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, northiaden, nortriptyline, pentobarbital, pheniramine, promethazine, propoxyphene, propranolol, protriptyline, quinidine, quinine, sulforidazine, thiothixene, tranlycypromine, trazodone, trihexiphenidyl, trimipramine, triprolidine

**Noninterfering:** dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, trifluoperazine

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**KEY WORDS**

serum; whole blood; liver

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**REFERENCE**

McIntyre, I.M.; King, C.V.; Skafidis, S.; Drummer, O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, 621, 215–223.

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**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50  $\mu$ L MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood)  $\mu$ L aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200–350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

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**HPLC VARIABLES**

**Guard column:** 20 mm long Symmetry C18

**Column:** 250  $\times$  4.6 5  $\mu$ m Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10–30

**Detector:** UV 262.9

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**CHROMATOGRAM**

**Retention time:** 17.168

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**KEY WORDS**

whole blood

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**REFERENCE**

Gaillard, Y.; Pépin, G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, 763, 149–163.

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**SAMPLE**

**Matrix:** formulations

**Sample preparation:** Crush tablet or capsule, to 2 mg amitriptyline add 20 mL MeOH, shake 30 min, centrifuge at 2000 rpm for 5 min, to 5 mL supernatant add 4 mL 1.25 mg/mL norephedrine.HCl in MeOH, dilute to 10 mL with MeOH.

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**HPLC VARIABLES**

**Column:** 150  $\times$  4.6 5  $\mu$ m Zorbax CN

**Mobile phase:** MeCN:MeOH:25 mM pH 4.8 sodium acetate-acetic acid buffer 35:45:20  
**Flow rate:** 2.5  
**Injection volume:** 10  
**Detector:** UV 254

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#### CHROMATOGRAM

**Retention time:** 5.7  
**Internal standard:** norephedrine (2.7)

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#### OTHER SUBSTANCES

**Also analyzed:** chlorpromazine, amitriptyline, imipramine, trifluoperazine

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#### KEY WORDS

tablets; capsules

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#### REFERENCE

Lovering, E.G.; Beaulieu, N.; Lawrence, R.C.; Sears, R.W. Liquid chromatographic method for identity, assay, and content uniformity of five tricyclic drugs, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 168–171.

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#### SAMPLE

**Matrix:** hair

**Sample preparation:** Wash hair in water, rinse 3 times with MeOH, dry, weigh. 5–25 mg Washed hair + 1 mL 1 M NaOH, heat at 70° for 30 min, adjust pH to 9.5–10. 1 mL Extract + 1 µg protriptyline + 1 mL water + 1 mL 200 mM sodium carbonate buffer, mix, extract with hexane: butanol 95:5 for 20 min. Remove the organic layer and add it to 100 µL 0.2% orthophosphoric acid, mix for 20 min, inject a 30 µL aliquot of the aqueous layer.

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#### HPLC VARIABLES

**Column:** 150 × 3.9 5 µm Nova-Pak phenyl  
**Mobile phase:** MeCN:buffer 55:45 (Buffer was 10 mM pH 3.0 KH<sub>2</sub>PO<sub>4</sub>.)  
**Flow rate:** 1.5  
**Injection volume:** 30  
**Detector:** UV 265

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#### CHROMATOGRAM

**Internal standard:** protriptyline (UV 214) (4)

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#### OTHER SUBSTANCES

**Extracted:** chlorpromazine (UV 255)

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#### REFERENCE

Couper, F.J.; McIntyre, I.M.; Drummer, O.H. Extraction of psychotropic drugs from human scalp hair, *J.Forensic Sci.*, **1995**, *40*, 83–86.

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#### SAMPLE

**Matrix:** microsomal incubations

**Sample preparation:** 1 mL Microsomal incubation + 2.5 mL MTBE:hexane 75:25, vortex for 20 s, centrifuge at 2000 g for 5 min, remove and retain the organic layer. Add 750 µL 1 M sodium carbonate, 200 µL 2 M NaOH, and 2.5 mL MTBE:hexane 75:25 to the aqueous layer, vortex for 20 s, centrifuge at 2000 g for 5 min. Repeat the extraction with 2.5 mL dichloromethane. Combine all the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase, inject an aliquot.

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#### HPLC VARIABLES

**Column:** 5 µm silica RCM 8 × 10 radial compression (Waters)  
**Mobile phase:** Isooctane:MeOH:dichloromethane 80:10:10 containing 0.036% methylamine  
**Flow rate:** 2  
**Detector:** UV 254

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#### CHROMATOGRAM

**Retention time:** 2.3

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**OTHER SUBSTANCES****Extracted:** metabolites**KEY WORDS**

mouse; liver; normal phase

**REFERENCE**

Blake, B.L.; Rose, R.L.; Mailman, R.B.; Levi, P.E.; Hodgson, E. Metabolism of thioridazine by microsomal monooxygenases: relative roles of P450 and flavin-containing monooxygenase, *Xenobiotica*, **1995**, *25*, 377-393.

**SAMPLE****Matrix:** solutions**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.**HPLC VARIABLES****Column:** 125 × 4.9 Spherisorb S5W silica**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7**Flow rate:** 2**Injection volume:** 20**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V**CHROMATOGRAM****Retention time:** 5.5**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclozine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, flupromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylegonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutaramide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenylamine, theophylline, thiethylperazine, thiopropazate, thiopropazine, thiothixene, thonzylamine, timolol, tocanide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

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**REFERENCE**

Jane, I.; McKinnon, A.; Flanagan, R. J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J. Chromatogr.*, **1985**, 323, 191–225.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Dissolve in MeOH:water 1:1 at a concentration of 50 µg/mL, inject a 10 µL aliquot.

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**HPLC VARIABLES**

**Column:** 300 × 3.9 10 µm µBondapak C18

**Mobile phase:** MeOH:acetic acid:triethylamine:water 70:1.5:0.5:28

**Flow rate:** 1.5

**Injection volume:** 10

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 9

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**OTHER SUBSTANCES**

**Simultaneous:** mesoridazine, promethazine, acetophenazine, chlorpromazine, prochlorperazine, butaperazine, thiethylperazine

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**REFERENCE**

Roos, R. W.; Lau-Cam, C. A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J. Chromatogr.*, **1986**, 370, 403–418.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Guard column:** 30 × 2.1 Spheri-5 RP-8

**Column:** 220 × 2.1 Spheri-5 RP-8

**Mobile phase:** Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.

**Column temperature:** 50

**Flow rate:** 0.5

**Detector:** UV 200

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**CHROMATOGRAM**

**Retention time:** 16.2

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**OTHER SUBSTANCES**

**Simultaneous:** mesoridazine, promazine, thiothixene, chlorpromazine, trifluoperazine

**Also analyzed:** amitriptyline, amphetamine, chlordiazepoxide, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, diethylpropion, doxepin, ephedrine, fenfluramine, flurazepam, imipramine, methamphetamine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, phentermine, phenylpropanolamine, prazepam

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**REFERENCE**

*Rainin Catalog*, C1-94, **1994**, p. 7.24.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Prepare a 1 mg/mL solution in MeOH, inject a 5 µL aliquot.

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**HPLC VARIABLES**

**Column:** 250 × 4.6 5 µm Lichrosphere cyanopropyl

**Mobile phase:** Carbon dioxide:MeOH:isopropylamine 90:10:0.05

**Column temperature:** 50

**Flow rate:** 3

**Injection volume:** 5

**Detector:** UV 220

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## CHROMATOGRAM

**Retention time:** 5.38

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## OTHER SUBSTANCES

**Simultaneous:** benactyzine, buclizine, hydroxyzine, perphenazine, amitriptyline, desipramine, imipramine, nortriptyline, protriptyline

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## KEY WORDS

SFC; pressure 200 bar

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## REFERENCE

Berger, T.A.; Wilson, W.H. Separation of drugs by packed column supercritical fluid chromatography. 2. Anti-depressants, *J. Pharm. Sci.*, **1994**, *83*, 287-290.

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## SAMPLE

**Matrix:** solutions

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## HPLC VARIABLES

**Column:** 250 × 4.6 Zorbax RX

**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

**Column temperature:** 30

**Flow rate:** 2

**Detector:** UV 210

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## OTHER SUBSTANCES

**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlor-diazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenpropofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxystyryl, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methypylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine,

pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyridylidone, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

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## REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

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## SAMPLE

**Matrix:** solutions

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## HPLC VARIABLES

**Column:** 250 × 4.6 Chirex 3022 (Phenomenex)

**Mobile phase:** Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 58:35:7 (EtOH/trifluoroacetic acid was premixed 20:1.)

**Flow rate:** 0.7-1

**Injection volume:** 20

**Detector:** UV 264

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## KEY WORDS

chiral;  $\alpha = 1.10$  for enantiomers

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## REFERENCE

Cleveland,T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J.Liq.Chromatogr.*, **1995**, *18*, 649–671.

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## SAMPLE

**Matrix:** solutions

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## HPLC VARIABLES

**Column:** 250 × 4.5  $\mu$ m LiChrospher 100 RP-8

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 60:25:15 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6

**Injection volume:** 25

**Detector:** UV 229

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## CHROMATOGRAM

**Retention time:** 9.82

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## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordi-azepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide,

ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazinol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

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**KEY WORDS**

details of plasma extraction

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**REFERENCE**

Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, 692, 103–119.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Inject a 20  $\mu$ L aliquot of a 25 ng/mL solution in pH 4.0 acetate/citrate buffer.

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**HPLC VARIABLES**

**Column:** 150  $\times$  0.32 3  $\mu$ m Hypersil C18

**Mobile phase:** MeCN:pH 4.0 acetate/citrate buffer 45:55

**Injection volume:** 20

**Detector:** UV

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**CHROMATOGRAM**

**Retention time:** 14

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**OTHER SUBSTANCES**

**Simultaneous:** chlorpromazine, methotrimeprazine (levomepromazine)

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**KEY WORDS**

microcolumn

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**REFERENCE**

Streel, B.; Ceccato, A.; Chiap, P.; Hubert, P.; Crommen, J. Injection-generated solvent and pH gradients for sample enrichment on injection of large volumes in microcolumn liquid chromatography, *Biomed. Chromatogr.*, **1995**, 9, 254–256.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Inject a 20  $\mu$ L aliquot of a 100–500  $\mu$ g/mL solution in mobile phase.

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**HPLC VARIABLES**

**Column:** 100  $\times$  4.6 5  $\mu$ m Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)



**Mobile phase:** MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60

**Flow rate:** 0.5–2

**Injection volume:** 20

**Detector:** UV 254

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#### CHROMATOGRAM

**Retention time:** k' 22.39

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#### OTHER SUBSTANCES

**Also analyzed:** amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, testosterone, tolfenamic acid, verapamil

**Noninterfering:** acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

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#### KEY WORDS

comparison with capillary electrophoresis

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#### REFERENCE

Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, 70, 2092–2099.

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#### SAMPLE

**Matrix:** urine

**Sample preparation:** Perform all procedures in subdued light. 10 mL Urine + 10  $\mu$ L 100  $\mu$ g/mL IS, lyophilize, extract residue with 3 mL MeOH by shaking for 15 min, repeat extraction twice, combine extracts and evaporate them to dryness under vacuum below 45°, dissolve residue in 2 mL 300 mM pH 7.2 phosphate buffer, extract three times with 3 mL dichloromethane, wash the combined organic layers twice with 2 mL phosphate buffer, twice with 2 mL water, dry over anhydrous sodium sulfate, evaporate to dryness, reconstitute with 100  $\mu$ L MeCN, inject a 10  $\mu$ L aliquot.

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#### HPLC VARIABLES

**Column:** 150  $\times$  4.6 3  $\mu$ m Spherisorb cyano

**Mobile phase:** 2,2,4-Trimethylpentane:dichloromethane:MeOH:diethylamine 82:10:8:0.1

**Flow rate:** 1.1

**Injection volume:** 10

**Detector:** UV 254

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#### CHROMATOGRAM

**Retention time:** 2.9

**Internal standard:** mesoridazine lactam (18)

**Limit of quantitation:** 50 ng/mL

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#### OTHER SUBSTANCES

**Simultaneous:** metabolites

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#### KEY WORDS

human; rat; dog; normal phase

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#### REFERENCE

Lin,G.; Hawes,E.M.; McKay,G.; Korchinski,E.D.; Midha,K.K. Metabolism of piperidine-type phenothiazine antipsychotic agents. IV. Thioridazine in dog, man and rat, *Xenobiotica*, **1993**, 23, 1059–1074.